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EXAMINER
LIU, SAMUEL W

ART UNIT	PAPER NUMBER
1653	

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Please find below and/or attached an Office communication concerning this application or proceeding.

FILE COPY

Office Action Summary	Application No. 09/998,619	Applicant(s) CONN ET AL.	
	Examiner Samuel W Liu	Art Unit 1653	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 June 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-20* is/are pending in the application.

4a) Of the above claim(s) *10-12* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-9 and 13-20* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Priority/Amendment

Preliminary amendment filed 11 November 2001 prior to patent examination has been entered. Applicants' statement of domestic priority has been acknowledged.

Election/Restrictions

Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-9 and 13-20, drawn to method of preparation troponin I proteins in *E.coli* and *in vitro* chemical modification of the expressed product, classified in class 435, subclass 69.1 and class 530, subclass 335, 402 and 412.

II. Claims 10-12, drawn to troponin I (chemically modified polypeptide complex), classified in class 530, subclass 300 and 402.

The inventions are distinct, each from the other because of the following reasons:

Invention I and II are related as process of making and product made. The inventions are distinct if either or both of the following can be shown: (1) that the process as claimed can be used to make other and materially different product or (2) that the product as claimed can be made by another and materially different process (MPEP § 806.05(f)). In the instant case, the processes as claimed (producing polypeptide using an *E.coli* expression system or /and chemical modification of sulfhydryl group of Cysteine residue) are alternative processes of producing modified troponin I polypeptides.

For the reasons given above and since the inventions have acquired a separate status in the art as shown by their different classification, restriction for examination purposes as indicated is proper.

During a telephone conversation with William Ramey on June 20, 2002 a provisional election was made with traverse to prosecute the invention of Group I, Claims 1-9 and 13-20. Affirmation of this election must be made by applicant in replying to this Office action. Claims 10 –12 are withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention. Therefore, the elected claims 1-9 and 13-20 are examined in this Office Action.

Specification/ Objections

The disclosure is objected to because of the following informalities:

(1) In Page 3, line 16, "rTroponin-I" should be spelled out in full for the first time. The same are for the term "AEX" in the same page, line 26; "MW Stds" in Page 4, line 13; "UF/DF" in Page 13, line 10; "LysC" in Page 4, line10; and "LC/MS" in Page 4, line 25.

(2) In Page 5, line 18, "(1-6M)" should be changed to "(1-6 M)"; the same correction should be made where needed throughout the specification.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-9 and 13-20are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for sulfitolyzing and separating naturally occurring Troponin I (TnI) polypeptide, does not reasonably provide enablement for recombinant (mutant) TnI polypeptide. The specification provides insufficient guidance and no working examples as to

how to construct, express, sulfitolyze and chromatograph the mutant polypeptide(s) or peptide(s). The specification does not enable a person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

Applicants are in possession of chemically protecting sulfhydryl side chains, deprotecting and chromatographically separating naturally occurring TnI polypeptide. Applicants are not in possession of chemically protecting sulfhydryl side chains, deprotecting and chromatographically purifying all mutant (recombinant) TnI polypeptide.

There would have been numerous mutants e.g. truncation (deletion), substitution and fusion, which are different and/or distinct from one another in biochemical and physical properties (including different molecular weight) that would have an impact on chromatographic elution steps which are polypeptide-type-dependent (for instance, different proteins require different elution buffers as well as different applied gradients for an effective elution).

In this regards, the application disclosure and claims have been compared per the factors indicated in the decision *in re* Wands 8 USPQ2d 1400, 1400 (Fed. Cir. 1998). These factors are considered when determining whether there is sufficient evidence to support a description that a disclosure does not satisfy the enablement requirement and whether any necessary experimentation is undue. The factors include but not limited to: 1) the nature of the invention; 2) the breath of the claims; 3) the predictability or unpredictability of the art; 4) the amount of direction or guidance presented; 5) the presence or absence of working examples; 6) the quantity of experimentation necessary; 7) the relative skill of those skilled in the art.

Each factor is addressed below on the basis of comparison of the disclosure, the claims and the state of the prior art in the assessment of undue experimentation.

(1) The scope of the claims/The nature of the invention:

Claims 1-9 and 13-20 of the instant application are directed to a method of sulfitolyzation and chromatography of the mutant (recombinant) TnI polypeptide and/or peptide. The claimed recombinant TnI represents a genus encompassing an unpredictable number of mutants, which are substitution, truncation and fusion (e.g. homologous fusion between TnI fragments and heterologous fusion between TnI and other polypeptide fragments). Thus, there would have been various elution profiles for various mutant polypeptide or peptide but which are not described in the application as to any of the particulars for protection and isolation via chromatographic separation.

There is insufficient guidance as to the varied chromatographic elution profiles which are governed by different and/or distinct mutant polypeptides sulfitolyzed. The mutated peptide point mutations, would result in obviously distinct elution profiles (see Figures 4 and 5 shown by Paleari, R. et al. (1999) *Clinic. Chem.* 45, 21-28). Truncated mutants or fusion polypeptide have varied lengths of the primary structure and are unpredictable as to structure, modes of protection via the process set forth in the specification, as well as chromatographic separation process and parameters (how and what are the charges to the separation parameters based on the differences in structure?). Chromatographic elution profile(s) for the mutant peptides are unpredictable.

Consequently, the application does not present a representative description of the genus of TnI proteins isolated by the currently claimed process of ion-exchange chromatography followed by hydrophobic interaction chromatography.

(2) The state of the prior art:

The general knowledge and level of skilled in the art do not supplement the omitted description because specific, not general, guidance is what is needed. The disclosure fails to provide working examples as to how to construct recombinant TnI polypeptide and how to select the recombinant type (i.e. what type of mutagenesis, e.g. substitution, deletion etc.) being subject to chromatography, the skilled artisan is required to perform undue experimentation in order to test, identify and isolate the desired TnI mutant(s) which are protected by sulfitolyzation during large scale purification, and then can be reversibly de-sulfitolyzed upon completion of purification.

(3) The quantity of experimentation necessary:

In the absence of working examples with regard to the above mentioned numerous mutant peptides, the unpredictability of the art, the lack of sufficient guidance in the specification, and the breadth of the claims, it would take undue trial and error to practice the claimed invention. A skilled artisan would be required to carry out a large body of tests to modify both sulfitolyzation reaction and chromatographic elution condition since both operational conditions are largely dependent upon TnI mutant folding structures which in turn determine negative charge distribution on the mutant protein surface.

(4) The unpredictability of the art:

Because there are numerous mutants of TnI polypeptide or peptide associated with different length and different number of sulfhydryl group needed to be protected, and associated with developing necessary elution profiles for purifying the desired TnI recombinants in the absence of factual indicia to the contrary. Sulfitolyzation introduces additional negative charge(s) to the mutant proteins. Recombinant TnI polypeptide includes, in addition to point mutant and

other types of mutations, (i) a fusion protein that may possess more than three sulfhydryl groups, and (ii) truncation mutant that may possess less than three sulfhydryl groups; thus, sulfitolyzation-introduced negative charges would be unpredictable in light of mutant sulfitolyzation. In this regard, therefore, separation of different polyionic chains of the recombinant products on chromatography would require different chromatographic parameters from those set forth for separating non-mutant sulfitolyzed TnI polypeptides in the current application, and would result in different or/and distinct elution profiles. Stubenrauch, K. et al have shown the distinct profiles of interaction of polyionic peptide with ion-exchange matrices (see Figure 1 of *J. Chromatog.* (2000) 737, 77-84). Regardless of truncation, point mutants also produce distinct elution profile (see Figure 4 shown by Paleari, R. et al. (1999) *Clinic. Chem.* 45, 21-28). Therefore, in the absence of knowledge and information of TnI mutants (recombinant), outcome of separation of the same is highly unpredictable.

(5) The relative skill of those in the art:

The general knowledge and level of skill in the art do not supplement the omitted description with respect to mutant TnI polypeptide and the biological activity thereof. It is noted that the chemical reaction condition set forth in the current disclosure for TnI sulfitolyzation cannot be directly applied to mutant TnI polypeptide or peptide because some mutants may not contain Cysteine residue which is subject to modification by thiol-specific compound tetrathionate, and that development of chromatographic elution for the mutants would be different from that for naturally occurring TnI peptide (see the rationale in the forgoing statement).

Furthermore, the instant application is directed to purification of biologically active TnI polypeptide rather than inactive thiol-modified TnI polypeptide(s); yet, many TnI mutants would be inactive or partially active absent factual indicia to the contrary. There is insufficient guidance as to which amino acid residue within the polypeptide can be deleted, substituted and whether the resulting TnI polypeptide would maintain the same activity and structure as wild type TnI polypeptide. Ngo *et al* teach that the amino acid positions within the polypeptide/protein that can tolerate change such as conservative substitution or no substitution, addition or deletion which are critical to maintain the protein's structure will require guidance (see Ngo et al., 1994, *The Protein Folding Problem and Tertiary Structure Prediction*, pp. 492-495). Given the lack of sufficient guidance and working examples, predicting what amino acid residue(s) or sequence changes can be made to still retain a functional fold is unpredictable and a skilled artisan is not able to supplement the omitted description regarding the mutant design, construction and selection followed by sulfhydryl group protection and chromatography.

In view of this regard and the preceding factors (1-4), the level of skill in this art is high and requires at least a biochemist at Ph.D. level with several years of experience in peptide chemistry, protein purification, molecular biology and biochemistry; yet, even with that level of skill in the art, predictability of the results is still highly variable.

In consideration of each of factors stated above, there is undue experimentation because of variability in prediction of outcome that is not addressed by the instant application disclosure, examples, teaching, and guidance presented. Absent factual data to the contrary, the amount and level of experimentation needed is undue.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter that the applicant regards as his invention.

Claims 1, 3, 4, 8, 9 and 13-20 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 is unclear as to what and with what the sulfhydryl groups are protected under reducing conditions, and whether or not they are only protected under reducing conditions or is it the reducing conditions that are necessary to effect the process of protecting group.

Claim 3 is unclear because there is no apparent antecedent basis in Claim2 or Claim 1 for "reduced recombinant" TnI. Claim 4 is also indefinite for lack of antecedent basis for reciting "the recombinant troponin I".

Claim 8 is indefinite as to whether or not the troponin I remains protected in the non-reducing conditions of Claim 8 since Claim 1 and 6 only indicate protection under reducing conditions. Claim 9 is indefinite as to the double period in line 2 (see "Troponin I..").

Claims 13 and 16 and claims dependent thereto are indefinite as to what is the result of subjecting the protein to a chromatography step.

Claim 15 recites "reacting reduced, denatured recombinant Troponin I with" which renders the claim indefinite because it is not apparent as to what is "reacting reduced" and whether or not sulfitolyzation comprises denatured recombinant Troponin I which is not reduced.

Claim 19 is indefinite because the recitation "chromatographic support" in chromatography refers to a material having an absorbent capability that is loaded onto the

chromatographic column. The recitation is not apparent as to whether or not the said chromatographic support is (a) a material or (b) a column, or a combination of (a) and (b). The dependent Claim 20 is also rejected.

Claim 20 is indefinite as being of improper dependent form for failing to further limit the subject matter of a previous claim. Note that there is no intrinsic relationship between Claim 20 and Claim 19 from which Claim 20 depends and that feature or characteristic set forth by the claims 19 and 20 are distinct, one is directed to ionic exchange chromatography (Claim19) whereas the other is directed to hydrophobic interaction chromatography. Applicant is required to amend the claim so that the claim is dependent from a proper claim or cancel the claim.

Claim Rejections - 35 USC §103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later

invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-9 and 13-20 are rejected under 35 U.S.C. 103(a) as being obvious over (1) Fujita-Becker, S. et al. (*J. Biochem.* (1993) 114, 438-444) taken with (2) Reiffert, S. et al. (*Eur. J. Biochem.* (1999) 261, 40-47); (3) Grushoff, P.S. et al. (*J. Bacteriol.* (1975) 122, 599-605); and (4) Hung C.-H., et al (US Patent SN: 4734362).

Fujita-Becker et al, teach purification of Troponin I (recited in Claim 1) from *E.coli* expression system, as applied to Claims 4-5 and 17-18 limitation of "TnI is expressed in an *E.coli* expression system", using Q-Sepharose column (anion-exchange column) and Phenyl-Sepharose column (a hydrophobic interaction chromatographic column) (Claim 19-20 limitation of "anion exchange column' and "hydrophobic interaction chromatographic support", respectively).

Insofar as Fujita-Becker et al. do not teach purifying recombinant TnI polypeptide set forth in Claims 3, 6 and 15 of the instant application. Reiffert et al. expressly teach purification of genetically engineered Troponin I protein from bacterial culture using ion-exchange and a reverse phase hydrophobic interaction column (e.g. HPLC C18 column, see "Materials and methods" section, page 41), as applied to Claims 3, 6 and 15 and dependent claims thereof.

Grushoff. et al teach chromatographic purification (ion-exchange, see page 108 and Figures 2 and 3) of a bacterial protein utilizing tetrathionate to protect the protein by blocking the sulfhydryl groups of the protein (see page 600, "Materials and Methods" section), which is applicable for limitations of Claims 1-3, 6-8 and 13-16; however, Grushoff et al do not teach

purification of Troponin I protein and recombinant thereof; but, Reiffert et al do teach so isolation and purification of troponin I.

Hung et al teach a deprotecting method i.e. daglocking protected sulfhydryl groups of the recombinant polypeptide (see columns 11-12 and Claims 32-37).

It would have been obvious to one of ordinary skill in the art at the time the invention was made would have combined the teachings of Fujita-Becker et al, Reiffert et al, Grushoff et al and Hung et al for the advantages of as follows: (1) obtaining homogenous preparation utilizing disodium tetrathionate to protect protein by blocking the sulfhydryl groups associated with two-step chromatography to purify biological active proteins (enzymes) taught by Grushoff et al. (see abstract and result section); (2) in vitro reconstitution of troponin complex which components are purified to highly homogeneous, stable, and obtainable in large quantities and also applicable for crystallization trials taught by Fujita-Becker et al (see abstract and result section); and (3) structural characterization of the purified recombinant TnI protein is carried out by isoelectic focusing, mass spectrometry and CD spectroscopy in order for in vitro reconstitution study taught by Reiffiert et al. (see abstract and result section).

Given the above motivation one of ordinary skill in the art would have combined (i) the teachings of Grushoff et al regarding use of tetrathionate to block the sulfhydryl groups of protein to facilitate chromatographic purification of a bacterially expressed protein, (ii) the teachings of Fujita-Becker et al and Reiffert et al with respect to purifying non-recombinant and recombinant TnI proteins by anion-exchange and hydrophobic interaction chromatography from *E.coli* expression system, and (iii) the teaching of US patent SN: 4734362 regarding deprotection of modified sulfhydryl groups in order to obtain final functional preparation, which when

combined it would have result in effective purification of bioactive sulfitolyzed TnI proteins from a *E.coli* expression system. Thus, as explained above the claimed invention was prima facie obvious to make and use the invention at the time it was made.

Claim Rejection, 35 U.S.C. 101, Double Patenting

A rejection based on double patenting of the "same invention" type finds its support in the language of 35 U.S.C. 101 which states that "whoever invents or discovers any new and useful process... may obtain a patent therefore..." (Emphasis added). Thus, the term "same invention," in this context, means an invention drawn to identical subject matter. See *Miller v. Eagle Mfg. Co.*, 151 U.S. 186 (1894); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Ockert*, 245 F.2d 467, 114 USPQ 330 (CCPA 1957).

A statutory type (35 U.S.C. 101) double patenting rejection can be overcome by canceling or amending the conflicting claims so they are no longer coextensive in scope. The filing of a terminal disclaimer cannot overcome a double patenting rejection based upon 35 U.S.C. 101.

Claims 1, 2, 4-14 and 16-20 are provisionally rejected under the judicially created doctrine of double patenting as being directed to the same invention as that set forth in claims 1-20 of copending Application NO: 09903398. Each claim in this application is word for word identical to the claims in the copending application. This is a provisional double patenting rejection because the claims have not in fact been patented.

Claim Rejections - Provisional Rejection, Obviousness Type Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or

improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130 (b). Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 3 and 15 of the instant application are provisionally rejected under the judicially created doctrine of double patenting over claims 3 and 15 of copending Application No. 09903398. This is a provisional double patenting rejection because the conflicting claims have not in fact been patented.

The subject matter claimed in the instant application is fully disclosed in the referenced copending application and would be covered by any patent granted on that copending application since the referenced copending application and the instant application are claiming common subject matter.

Claims 3 and 15 of Application 09903398 sets forth using sodium sulfite (Na_2SO_3), which is same type of inorganic sulfhydryl-blocking reagent as tetrathionate ($Na_2S_4O_6$) set forth by Claim 3 and 15 of the instant application. Note that both sulfite ion and tetrathionate ion have two negative charges with the latter being more specific compound in use for modification

of sulfhydryl group than the former. In view of the sulfhydryl-protecting function, these two reagents are expected to function identically; and, given the target peptides have same number of Cysteine residue(s), the sulfitolyzed peptides would be eluted under similar chromatographic gradient or condition. Therefore, the parent and copending application claims are obvious variation.

Claims 1, 2, 4-14 and 16-20 of this application conflict with claims 1-20 of Application NO: 09903398. 37 CFR 1.78(b) provides that when two or more applications filed by the same applicant contain conflicting claims, elimination of such claims from all but one application may be required in the absence of good and sufficient reason for their retention during pendency in more than one application. Applicant is required to either cancel the conflicting claims from all but one application or maintain a clear line of demarcation between the applications. See MPEP § 822.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Samuel Wei Liu whose telephone number is (703) 306-3483. The examiner can normally be reached from 9:00 a.m. to 5:00 p.m. on weekdays. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dr. Christopher Low, can be reached on 703 308-2923. The fax phone number for the organization where this application or proceeding is assigned is 703 308-4242 or 703 872-9306 (official) or 703 872-9307 (after final). Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703 305-4700.

SWL

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June 18, 2002

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